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(54) Title: METHODS OF IDENTIFYING AND DIAGNOSING INFLAMMATORY BOWEL DISEASE

(57) Abstract

The present invention provides methods of identifying, diagnosing and screening for inflammatory bowel disease, particularly Crohn's Disease (CD) comprising identifying alleles and polymorphisms associated with a biological response related to an inflammatory bowel disease. The invention further provides for a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The invention also provides for an assay system for screening for susceptibility to inflammatory bowel disease.

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METHODS OF IDENTIFYING AND DIAGNOSING INFLAMMATORY BOWEL DISEASE

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BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

This invention relates generally to the field of identifying, diagnosing and screening for inflammatory bowel disease, and more specifically, to methods of identifying, diagnosing and screening for Crohn's Disease.

BACKGROUND INFORMATION

Inflammatory Bowel Disease

Inflammatory Bowel Disease ("IBD") is the collective term used to describe two chronic, idiopathic inflammatory diseases of the gastrointestinal tract: ulcerative colitis ("UC") and Crohn's disease ("CD"). IBD occurs world-wide and is reported to afflict as many as two million people. UC and CD are grouped together because of their overlapping clinical, etiologic, and pathogenic features. Furthermore, clinical data subtyping the disease provides evidence that CD may be a heterogenous group of disorders, although the etiologies and pathogeneses of these CD subtypes remain unknown. Targan, S., *et al., Nature Med.* 1:1241-1242 (1995). From a therapeutic and prognostic standpoint, however, it is as important to distinguish between both UC and CD and between subtypes of CD as it is to distinguish chronic from non-chronic inflammatory diseases of the bowel. The heterogeneity underlying CD, for example, can be reflected in the variable responses of CD patients to a particular treatment strategy. The availability of methods of diagnosing a clinical subtype of CD would represent a major clinical advance that would aid in the therapeutic management of CD and would provide a basis for the design of treatment modalities that are specific to a particular disease subtype. Unfortunately, a method of

stratifying CD into clinical subtypes to allow the design of more precise treatment strategies is currently not available. Thus, there is a need for a method of diagnosing a clinical subtype of CD. The present invention satisfies this need and provides related advantages as well.

The Causes of IBD are Unknown

Although the causes of UC and CD are not known, there is general agreement that genetics is important in a person's susceptibility to IBD and that the immune system is responsible for mediating tissue damage in these diseases. Generally speaking, a failure to down-regulate the normal, self-limited inflammatory responses in the bowel is a characteristic of IBD. While a wide range of immunologic abnormalities and dysfunctions have been reported in these disorders, before this invention none had yet been associated with specific TNF alleles or polymorphisms to be of diagnostic or prognostic value.

A battery of laboratory, radiological, and endoscopic evaluations are combined to derive a diagnosis of IBD and to assess the extent and severity of the disease. Nevertheless, differentiating UC from CD, as well as other types of inflammatory conditions of the bowel such as irritable bowel syndrome, infectious diarrhea, rectal bleeding, radiation colitis, and the like, is difficult, because the mucosa of the small and large intestines react in a similar way to a large number of different conditions. Once infectious-types of bowel disorders have been ruled out, the final diagnosis of IBD is often made on the basis of the progression of the disease. In many patients, though, a diagnosis must still be regarded as indeterminate because of the overlapping features of UC and CD, particularly with CD of the colon. Thus, a great need exists for tools which can be used to identify CD heterogeneities and diagnose clinical CD subtypes.

Need for Objective Diagnostic Tools

To date there exist no objective diagnostic tools for the identification and diagnosis of subtypes of CD. Diagnosis

depends upon a host of procedures aimed at confirming the suspected diagnosis of CD. The initial symptoms are often confused for non-chronic bowel disorders by physicians unfamiliar with IBD. Consequently, IBD often goes mistreated and undiagnosed until the disease shows its chronicity which results in referral of the patient to a specialist. The imprecise and subjective nature of endoscopic and radiologic examination can result in a misdiagnosis between UC and CD, indeterminate diagnosis of CD. At present the physician has no means to identify and diagnose clinical subtypes of CD.

Histological examination does provide greater certainty of an accurate diagnosis, but the problems of differentiating between UC and CD based on histological findings are often underestimated. There is no single histological criterion which definitively identifies CD or UC or subtypes of CD. For example, the epithelial cell granuloma, which is often accorded a key role in the diagnosis of CD, is only found in about 20% of biopsy specimens taken from patients diagnosed with CD.

The patient often suffers as the disease progresses before a definitive diagnosis can be made. The selective identification of CD as opposed to UC or other inflammatory conditions of the intestines carries important prognostic and therapeutic implications. For example, when colectomy is indicated, the type of IBD involved determines which surgical options are appropriate. While in UC total colectomy surgery represents somewhat of a cure, in CD surgery is never curative. Continent procedures such as the ileorectal pull-through (mucosal proctectomy) or the Kock pouch may be desirable in UC, but are contra-indicated in CD.

The availability of genetic markers and corresponding immunological markers that would readily distinguish between CD from UC and identify subtypes of CD, either independent of or in combination with existing diagnostic tools, would represent a major clinical advance which would aid in therapeutic management of IBD and the design of more specific treatment modalities. Accordingly, there exists a need for

convenient and reliable methods of screening for subtypes of CD and distinguishing CD from UC for diagnostic, prognostic and therapeutic purposes. This invention satisfies these needs and provides related advantages as well.

Increased Mucosal TNF-α Levels and Numbers of TNF-α Producing Cells are Unique to Crohn's Disease

Tumor necrosis factor-α (TNF-α) is a T-cell and monocyte derived cytokine. It can mediate immunological and inflammatory events in Crohn's disease. Increased TNF-α messenger RNA levels are found in lamina propria mononuclear cells (LPMC) from inflamed CD mucosa. Spontaneous TNF-α protein production has been measured using a reversed ELISA spot assay and TNF-α mRNA levels has been measured using competitive reverse transcriptase-polymerase chain reaction (RT-PCR) from LPMC from CD, UC and control mucosa. Elevated levels of TNF-α mRNA and increased numbers of TNF-α producing cells are found exclusively in LPMC from inflamed CD, but not in uninflamed CD, inflamed or uninflamed UC or normal intestinal mucosa. Targan, S.R., *et al.*,*Gastroenterology* 106:A754 (1994). Plevy, S.E., *et al.*, *J. Immunol.* 150, no. 8, page 10A, abst. 41 (1993). Plevy, S.E., *et al.*, *FASEB J.* vol. 8, no. 4-5, abst. 5849, page A1010 (1994).

However, before this invention the suggestion that an immunologic abnormality in CD has a genetic determinant which would be of value in the diagnosis, screening or prognosis of an inflammatory bowel disease, such as CD or a subtype of CD, would have necessarily been speculative in nature.

SUMMARY OF THE INVENTION

The present invention provides methods of identifying, diagnosing and screening for IBD, particularly Crohn's Disease (CD), comprising identifying alleles or an allelic combination and polymorphisms associated with a biological response related to an inflammatory bowel disease. The invention further provides for a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The invention also provides for an assay system for screening for the diagnosis or susceptibility to inflammatory bowel disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic of a portion of Chromosome 6 demonstrating the mapping of the TNF locus; specifically, the microsatellite loci approximate the TNF-α and TNF-β loci.

Figure 2A demonstrates that total TNF production from lectin (Con A) and phorbol ester-activated PBMC of CD patients is as a whole greater than TNF production in UC patients. Figure 2B demonstrates that TNF microsatellite haplotype a2b1c2d4e1 correlates with increases in TNF-α protein production in CD. Figure 2C demonstrates that patients who had the TNF haplotype a2b1c2d4e1, when compared patients who had none of these individual alleles, had increased TNF responses to Con A and PMA stimulation.

Figure 3 demonstrates that a particular allele within a microsatellite haplotype is associated with increased TNF production in CD.

Figure 4 demonstrates that the TNF microsatellite haplotype a2b1c2d4e1 is not linked to all TNF-α dysregulated allelic variations in CD patients.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of identifying, diagnosing and screening for inflammatory bowel disease, particularly Crohn's Disease (CD). These methods include identifying an inflammatory bowel disease or a subtype thereof in a subject comprising: detecting an allele or an allelic

combination at a TNF locus by analyzing a nucleic acid from a subject; identifying a biological response related to the inflammatory bowel disease; and, identifying a correlation between the allele or allelic combination and the biological response. A further embodiment includes a method of diagnosing an inflammatory bowel disease in a subject comprising: detecting an allele or an allelic combination at a TNF locus by analyzing a nucleic acid from a subject; and, using a correlation found by the method of claim 1 to diagnose the inflammatory bowel disease. A further embodiment includes a method of screening for a susceptibility to an inflammatory bowel disease in a subject comprising: detecting an allele or an allelic combination at a TNF locus by analyzing a nucleic acid from a subject; and, using a correlation found by the method of claim 1 to evaluate the susceptibility of the subject for the inflammatory bowel disease. In a preferred embodiment, the inflammatory disease is Crohn's Disease, the TNF locus is the TNF-α locus, and the TNF-α locus allele is the d4 allele.

The invention also provides for a method of identifying an inflammatory bowel disease or a subtype thereof in a subject comprising: detecting a polymorphism at a TNF locus by analyzing a nucleic acid from a subject; identifying a biological response related to the inflammatory bowel disease; and, identifying a correlation between the polymorphism and the biological response. A further embodiment includes a method of diagnosing an inflammatory bowel disease in a subject comprising: detecting a polymorphism at a TNF locus by analyzing a nucleic acid from a subject; and, using a correlation found by the method of claim 1 to diagnose the inflammatory bowel disease. A further embodiment includes a method of screening for a susceptibility to an inflammatory bowel disease in a subject comprising: detecting a polymorphism at a TNF locus by analyzing a nucleic acid from a subject; using a correlation found by the method of claim 1 to evaluate the susceptibility of the subject for the inflammatory bowel disease. The polymorphism can comprise any

nucleotide substitution, addition, deletion or combination thereof. In a preferred embodiment, the nucleotide substitution comprises a substitution in the 5' regulatory region of a TNF-α loci. In another preferred embodiment, the nucleotide substitution in said TNF-α loci 5' regulatory region is selected from the group consisting of a guanosine nucleotide substitution for adenosine at the -238 position and a guanosine nucleotide substitution for adenosine at the -308 position. A preferred embodiment is a method of screening for a susceptibility to Crohn's Disease in a subject comprising detecting a d4 allele at a TNF locus by analyzing a nucleic acid from the subject.

The invention further provides for a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The invention also provides for an assay system for screening for a susceptibility to an inflammatory bowel disease in a subject comprising nucleic acid encoding a d4 allele. In another embodiment the assay system further comprising one or more nucleic acid primers specific for amplification of nucleic acid encoding a d4 allele. As a preferred embodiment the assay system screens for Crohn's Disease.

As disclosed herein, the methods of the invention can be further used to identify new genetic marker correlations that can identify new subtypes and new categories of various inflammatory bowel diseases, particularly new clinical subtypes of Crohn's disease.

In the following description, reference will be made to various methodologies known to those skilled in the art of molecular genetics, microbiology, pathology and general biology. Publications and other materials setting forth such known methodologies to which reference is made are incorporated herein by reference in their entireties as though set forth in full.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. In

the description that follows, a number of terms used in biotechnology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

As used herein, the term "inflammatory bowel disease" means any disease of the bowels with an infectious, inflammatory, autoimmune or hyperimmune component or response, acute or chronic, including ulcerative colitis and Crohn's disease (CD).

As used herein, the term "subject" refers to any animal, particularly a mammal, more particularly a human.

As used herein, the term "diagnosed with having an inflammatory bowel disease" refers to any diagnoses, whether based on symptoms, empirical data or the like, whether tentative or definitive.

As used herein, the term "allelic combination" means the same as a "haplotype," *i.e.* is referring to particular combination of alleles for at least two loci.

As used herein, the term "locus" means a physical location, place or position occupied by a particular gene on a chromosome.

As used herein, the term "TNF locus" refers to any DNA or chromosomal segment encoding for a TNF or which is structurally or functionally associated with or influencing the expression of any TNF gene.

As used herein, the term "related to" refers to a finding that the probability that at least two occurrences happen together or two things co-exist are greater than would be expected by chance alone.

As used herein, the term "susceptibility" refers to a subject having any increased chance or risk, *i.e.* increased probability, of an occurrence, such as acquiring a disease or condition, over what would be expected by chance alone.

As used herein, the term "screening" refers to any evaluation of a subject for susceptibility to a disease or condition, for example, by identifying any correlation between

a genetic profile, an allele, an allelic combination, or a polymorphism and an increased chance of acquiring a disease or condition, *i.e.* whether a subject is susceptible to acquiring a disease or condition. The term screening also refers to methods of diagnosis.

As used herein, the terms "identifying" and "detecting" refer to any means of detecting or identifying, for example, including hybridization with specific primers or probes and detection of such specific hybridizations, DNA sequencing, antibody binding and detection, or the like. The detecting can also include the use of isotopic or non-isotopic detection, such as fluorescent detection or enzyme labeled-oligonucleotides or a hybridization protection assay.

As used herein, the term "biological response" refers to any cellular, neurological, chemical, inflammatory, immunologic or pathologic biological response, process or reaction by the subject. The response, process or reaction can be chemical, cellular, neurological, psychological or the like.

As used herein, the term "increase in the levels of a cytokine" means any increase in the expression of a cytokine, such as TNFs. For example, increases in the level of TNF expression includes increases in TNF-α and TNF-β expression. The increases can be due to greater rates of mRNA transcription, greater rates of mRNA translation, longer mRNA or protein half-lifes and the like.

As used herein, the term "a therapy which decreases the levels of TNF-α" means any therapy which decreases the expression, effective concentration or bioavailability of the cytokine. For example, a therapy can decrease the level of TNF expression by decreasing rates of mRNA transcription or translation. The therapy can shorten mRNA or protein half-life. The therapy can neutralize the cytokine's activity, increase its clearance, compartmentalize it, and the like. The therapy can indirectly effect the levels of the cytokine by decreasing the numbers or activity of cell which secrete the cytokine.

As used herein, the term "allele" means alternative genes that occupy the same chromosomal locus, with an alternative gene including any modification or variation of a gene.

As used herein, the term "polymorphism" means the occurrence of two or more forms, such as the different forms of a nucleic acid in individuals of the same species. For example, the different form can be a difference in DNA sequences between individuals due to a substitution, a deletion or an addition.

The term "nucleic acid" as used herein means a polynucleotide such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). A nucleic acid can be either single-stranded or double-stranded. To practice the methods of the invention, a particularly useful nucleic acid is genomic DNA, complementary DNA or messenger RNA. The methods and assay systems of the invention employ nucleic acid. Nucleic acid of a subject which is suitable for manipulation, such as for diagnoses or screening, in accordance with the present invention may be derived from any nucleated cell sample, and preferably from peripheral mononuclear blood cells.

As used herein, the term "under conditions suitable for formation of a specific hybrid" means any set of physical conditions (such as temperature) or chemical conditions (such as pH, salt concentration) wherein an oligonucleotide probe or primer will form a hydrogen bonded, sequence-specific association with, i.e. specifically hybridize to, the DNA target sequence (such as an allele) to which the probe or primer nucleotide sequence is complementary. Defining such parameters and conditions is routine to one skilled in the art, and for example is described in Sambrook *et al.* and Mullis *et al.*, both of which have been incorporated by reference.

As used herein, the term "amplification" refers to the generation of specific DNA sequences using specific oligonucleotide primers through the use of techniques generally described as polymerase chain reaction (PCR), which are well known in the art, described for example in *PCR*

Technology, Erlich, Ed., Stockton Press, New York, N.Y, (1989) incorporated herein by reference; and, Sambrook *et al.* and Mullis *et al.*, both of which have been incorporated by reference.

As used herein, the term "assay system" means any kit or automated system, any form or combination that the reagents of the methods described herein can be combined, formulated or utilized. The assay system includes any combination of methods and reagents, manual or automated, including robotic systems, for the diagnosis of IBD, specifically CD, and for screening for a susceptibility to CD.

Detecting or identifying the presence or absence of nucleic acid of a subject encoding TNF alleles and polymorphisms associated with CD may be accomplished by any means. One of skill in the art will understand that there are many means available to make such a determination, *e.g.*, electrophoresis, automated sequencing, allele-specific oligonucleotide probing, differential restriction endonuclease digestion, ligase-mediated gene detection, and the like.

Inflammatory bowel disease has been classified into the broad categories of Crohn's Disease (CD) and ulcerative colitis (UC). CD, also called regional enteritis, is a disease of chronic inflammation that can involve any part of the gastrointestinal tract. Commonly the distal portion of the small intestine (ileum) and cecum are affected. In other cases, the disease is confined to the small intestine, colon or anorectal region. CD occasionally involves the duodenum and stomach, and more rarely the esophagus and oral cavity.

The variable clinical manifestations of CD are, in part, a result of the varying anatomic localization of the disease. The most frequent symptoms of CD are abdominal pain, diarrhea and recurrent fever. CD is commonly associated with intestinal obstruction or fistula, which is an abnormal passage between diseased loops of bowel, for example. CD also includes complications such as inflammation of the eye, joints and skin; liver disease; kidney stones or amyloidosis. In

addition, CD is associated with an increased risk of intestinal cancer.

Several features are characteristic of the pathology of CD. The inflammation associated with CD, known as transmural inflammation, involves all layers of the bowel wall. Thickening and edema, for example, typically also appear throughout the bowel wall, with fibrosis also present in long-standing disease. The inflammation characteristic of CD also is discontinuous in that segments of inflamed tissue, known as "skip lesions," are separated by apparently normal intestine. Furthermore, linear ulcerations, edema, and inflammation of the intervening tissue lead to a "cobblestone" appearance of the intestinal mucosa, which is distinctive of CD.

A hallmark of CD is the presence of discrete aggregations of inflammatory cells, known as granulomas, which are generally found in the submucosa. About half of CD cases display the typical discrete granulomas, while others show a diffuse granulomatous reaction or nonspecific transmural inflammation. As a result, the presence of discrete granulomas is indicative of CD, although the absence granulomas also is consistent with the disease. Thus, transmural or discontinuous inflammation, rather than the presence of granulomas, is a preferred diagnostic indicator of CD (Rubin and Farber, *Pathology* (Second Edition) Philadelphia: J.B. Lippincott Company (1994), which is incorporated herein by reference).

Ulcerative colitis (UC) is a disease of the large intestine characterized by chronic diarrhea with cramping abdominal pain, rectal bleeding, and loose discharges of blood, pus and mucus. The manifestations of UC vary widely. A pattern of exacerbations and remissions typifies the clinical course of most UC patients (70%), although continuous symptoms without remission are present in some patients with UC. Local and systemic complications of UC include arthritis, eye inflammation such as uveitis, skin ulcers and liver disease. In addition, UC and especially long-standing, extensive

disease is associated with an increased risk of colon carcinoma.

In comparison with CD, which is a patchy disease with frequent sparing of the rectum, UC is characterized by a continuous inflammation of the colon that usually is more severe distally than proximally. The inflammation in UC is superficial in that it is usually limited to the mucosal layer and is characterized by an acute inflammatory infiltrate with neutrophils and crypt abscesses. In contrast, CD affects the entire thickness of the bowel wall with granulomas often, although not always, present. Disease that terminates at the ileocecal valve, or in the colon distal to it, is indicative of ulcerative colitis, while involvement of the terminal ileum, a cobblestone-like appearance, discrete ulcers or fistulas suggest CD. Characteristics that serve to distinguish CD from UC are summarized in Table 1 (Rubin and Farber, *supra*, 1994).

Table 1		
Feature	Crohn's Disease	Ulcerative Colitis
Macroscopic		
Thickened bowel wall	Typical	Uncommon
Luminal narrowing	Typical	Uncommon
"Skip" lesions	Common	Absent
Right colon predominance	Typical	Absent
Fissures and fistulas	Common	Absent
Circumscribed ulcers	Common	Absent
Confluent linear ulcers	Common	Absent
Pseudopolyps	Absent	Common
Microscopic		
Transmural inflammation	Typical	Uncommon
Submucosal fibrosis	Typical	Absent
Fissures	Typical	Rare
Granulomas	Common	Absent
Crypt abscesses	Uncommon	Typical

As used herein, "patient with Crohn's disease" is synonymous with the term "a subject diagnosed with having an inflammatory bowel disease" wherein the IBD is CD, and means a patient having a characteristic feature from at least two of the following categories: clinical, endoscopic, radiographic and histopathologic. As used herein, a characteristic clinical feature is perforating or fistulizing disease; or an obstructive symptom secondary to small bowel stenosis or stricture. As used herein, a characteristic endoscopic feature is a deep linear or serpiginous ulceration; a discrete ulcer in normal-appearing mucosa; cobblestoning; or discontinuous or asymmetric inflammation. As used herein, a characteristic radiographic feature is segmental disease (skip lesion); a small bowel or colon stricture; stenosis or

fistula. As used herein, a characteristic histopathologic feature is submucosal or transmural inflammation; multiple granulomas; marked focal cryptitis or focal chronic inflammatory infiltration within and between biopsies; or a skip lesion, including histologic rectal sparing in the absence of local therapy.

Patients with chronic inflammatory bowel disease generally are characterized as having either Crohn's disease or ulcerative colitis to describe specific patterns of disease, to predict outcomes based on expected natural histories, and to help guide medical and surgical treatment strategies. Clinical, endoscopic, and histopathologic criteria, as discussed above, have been developed to classify patients into one or the other category. However, overlap between CD and UC also has been demonstrated at a variety of levels by clinical, immunological and genetic studies, for example. Furthermore, CD and UC each can encompass a number of distinct conditions affecting the gastrointestinal tract, with different clinical subtypes being classified together as CD or UC because they present with similar symptoms. One embodiment of the present invention is directed to the discovery that such clinical subtypes of CD can be diagnosed by identifying a TNF locus allele or allelic combination or a TNF polymorphism that is correlated with a biological response related to a inflammatory bowel disease. The invention further provides for a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease.

In summary, the present invention provides non-invasive methods to diagnose, screen for and distinguish clinical subtypes of CD from UC, and assay systems to accomplish the same.

The invention also demonstrates, using nonparametric linkage methods, that there is a genetic linkage of CD to the TNF locus (nonparametric statistics are statistical calculations that are not based on any prior assumptions with respect to the variable and the probability distribution of the data).

Previously, only "genetic associations" between the TNF locus and CD has been shown. Example I demonstrates an analysis of 29 multiplex families by several nonparametric linkage methods supporting linkage of CD to the TNF locus. The results demonstrate that at least one gene involved in the pathogenesis of CD is located on the short arm of chromosome 6 which contains the human major histocompatibility complex (MHC).

In accordance with the present invention, also provided are methods of screening for CD comprising detecting the presence or absence of nucleic acid of a subject encoding TNF alleles and polymorphisms associated with CD, wherein the presence of nucleic acid encoding the allele or the polymorphism is indicative, *i.e.* predictive, of CD. The alleles can be TNF microsatellite alleles. TNF microsatellite alleles associated with CD include the a2, b1, c2, d4 and e1 TNF microsatellite alleles.

Nucleic acid encoding TNF alleles and polymorphisms associated with CD can be identified or detected in accordance with the present invention by amplifying the nucleic acid and identifying the TNF alleles and polymorphisms. TNF alleles can be identified by assaying nucleic acid of a subject for defining characteristics of TNF polymorphisms or alleles associated with CD and comparing the results to a positive and or negative control. Defining characteristics of nucleic acid encoding TNF alleles and polymorphisms associated with CD include, for example, size, sequence, type of sequence repeats, and the like. One skilled in the art would be able to isolate and sequence DNA from any region of the chromosome, such as from the MHC and the TNF loci. One skilled in the art would be able to identify primers suitable for use in amplifying and sequencing any particular nucleic acid using published sequence databanks. For example, the map and sequence of the human MHC and TNF locus is available from Genbank as part of the human genome project, NCBI, NIH, easily searchable, for example, on the internet world wide web at http://www.ncbi.nlm.nih.gov. NCBI's database is herein

incorporated by reference in its entirety. Primers suitable for use in hybridizing, amplifying or sequencing nucleic acid encoding TNF locus DNA, TNF alleles, particularly the d4 allele, are also provided in Plevy, S.E., *et al.*, PCT US95/06107, WO 95/31575, which is herein incorporated by reference in its entirety. Methods for identifying, isolating, manipulating, sequencing and analyzing DNA from patient samples are well known in the art, for example, see Molecular Cloning, 2nd Ed., Sambrook, J., *et al.*, Eds., Cold Spring Harbor Laboratory Press, Plainview, NY (1989); and, The Polymerase Chain Reaction, Mullis, K.B., *et al.*, Eds., Maple Press Co., York, PA, each of which are incorporated herein by reference in their entirety.

An allele or polymorphism at a polymorphic locus, such as the TNF loci, can be identified or detected by a variety of methods including assays using the polymerase chain reaction (PCR). Oligonucleotide hybridization, such as allele-specific hybridization (see Mullis et al., *Id.*, incorporated by reference), denaturing gradient gel electrophoresis (see, for example, Innis et al. (eds.), *PCR Protocols: A Guide to Methods and Application,* Academic Press, Inc., San Diego, CA (1990)) and restriction fragment length polymorphism based methods (Sambrook, *supra*, (1989)), which are well known in the art and encompassed within the invention.

Human TNF locus microsatellites were mapped, characterized and reported in Nedospasov, S.A., *et al.*, *J. Immunology*, 147:1053-1059 (1991); Jongeneel, C.V., *et al.*, *Proc. Natl. Acad. Sci. USA* 88:9717-9721 (1991); Udalova, I.A., *et al.*, *Genomics* 16:180-186 (1993), and Pociot, F., *et al.*, *Eur. J. Immun.* 23:224-231 (1993), each of which are incorporated herein by reference in their entirety. These TNF microsatellites are characterized by a series of CA/GT or CT/CA dinucleotide sequence repeats. Figure 1 depicts a map of the TNF microsatellite loci at the TNF locus. For a more detailed description of the TNF microsatellite loci and the TNF locus, see Nedospasov, S.A., *et al.*,; Jongeneel, C.V., *et al.*; Udalova, I.A., et *al.*; and, Pociot, F., et *al.*, as

indicated above, where each has been incorporated by reference.

Primer pairs suitable for use in the practice of the present invention are linear oligonucleotides ranging in length from about ten to about thirty nucleotides in length. One of the primers in the pair should be complementary to a nucleotide sequence upstream of the nucleic acid encoding the TNF locus targeted for amplification. The other primer should be complementary to a sequence located down stream of this target site. Preferably, the primers suitable for use in the present invention are specific for amplification of nucleic acid encoding the TNF locus do not prime amplification of nucleic acid which does not encode the TNF locus.

The sequence complementary to the primer pairs may be separated by as many nucleotides as the PCR technique and the other technique(s) for detecting the presence or absence of TNF alleles or polymorphisms associated with CD will allow, provided that an appropriate control is used. For example, if the presence or absence of nucleic acid of a subject encoding the TNF locus associated with CD is detected on the basis of size, then the primers used for amplification must not include amplification of nucleic acid flanking the allele which would interfere with the ability to detect polymorphic size differences (by inclusion, for example, of polymorphic size differences which may be present in regions flanking the TNF locus).

Primers suitable for use in amplifying nucleic acid encoding the TNF locus can be constructed using the oligonucleotide primer sequences described in Plevy, S.E., *et al.*, PCT US95/06107, WO 95/31575, the cell lines described in Udalova, I.A., *et al.*, *Genomics*, 16:180-186 (1993) and deposited with the ASHI and the CEPH, and the map and sequence of the TNF locus available from Genbank, NIH (all references have been incorporated herein by reference).

Assay systems for use in screening for CD and distinguishing CD from UC are also provided by the present invention. Such assay systems can include all or some of the positive

controls, negative controls, reagents, primers, sequencing markers, probes and antibodies described herein for determining the presence or absence of nucleic acid encoding TNF alleles or polymorphisms associated with CD. Assay systems of the present invention may contain, for example: nucleic acid encoding TNF alleles or polymorphisms associated with CD; nucleic acid encoding TNF locus, including TNF alleles, not known to be associated with CD; the nucleic acid sequence of TNF alleles or TNF polymorphisms; one or more labeled oligonucleotide probes specific for a particular TNF locus allele or polymorphism; one or more primers for amplification of nucleic acid encoding TNF DNA, such as TNF alleles or polymorphisms; reagents commonly used for amplification; polymerases such as DNA or reverse transcriptase DNA polymerase; antibody specific for, or which binds particular TNF alleles or TNF polymorphisms; and, combinations of any of the above.

As amenable, these suggested assay system components may be packaged in a manner customary for use by those of skill in the art. For example, these suggested assay system components may be provided in solution or as a liquid dispersion or the like. The assay system of the invention can be in the form of semi-automated or entirely automated systems, including robotic systems, for the diagnosis or for the evaluation for a susceptibility to disease, particularly CD.

A presently preferred embodiment of the inventive assay systems for use in screening for CD or distinguishing CD from UC comprises DNA encoding two or more TNF alleles or polymorphisms associated with CD in Tris-EDTA buffer solution preferably kept at 4°C or lyophilized. For example, TNF locus alleles can be selected from a group comprising the a2, b1, c2, d4 and e1 TNF microsatellite alleles.

Another embodiment of the inventive assay systems for use in screening for CD or distinguishing CD from UC further comprises one or more primers specific for amplification of nucleic acid encoding TNF microsatellite alleles, for example,

primers as described in Plevy, S.E., *et al.*, PCT US95/06107, WO 95/31575 (which has been incorporated by reference).

Yet another embodiment of the inventive assay systems for use in screening for CD or distinguishing CD from UC further comprises sequencing markers ranging in size from about 80 to 200 base pairs.

The invention will now be describe in greater detail by reference to the following non-limiting examples. The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

To demonstrate a genetic linkage of CD to the TNF locus, the invention provides for an analysis of 29 multiplex families by several nonparametric linkage methods.

A genetic association has been reported in CD using groups of polymorphic microsatellite markers at or near the TNF locus within the MHC on the short arm of human chromosome 6. However, a showing of a "genetic association" cannot distinguish between the possibilities that the MHC region actually contains genes that contribute to the pathogenesis of CD or simply whether the "association" results from population stratification. To distinguish between these possibilities, a linkage study was performed which tests whether a disease cosegregates in families with the marker locus and therefore is independent of specific alleles.

Genomic DNA from 29 families with two or more siblings affected with CD was obtained from the IBD cell bank at Cedars-Sinai Medical Center, Los Angeles, CA. TNF microsatellite alleles at five loci: a, b, c, d, e were typed by polymerase chain reaction (PCR), see Plevy, S.E., *et al.*, PCT US95/06107, WO 95/31575, which is herein incorporated by reference in its entirety. Alleles were interpreted independently by two investigators blinded to diagnosis and family pedigrees. As there is no clear pattern of Mendelian inheritance for CD, nonparametric linkage analyses which do not assume a mode of inheritance were employed. The SIBPAL program from the S.A.G.E. package was used for affected

sibpair analysis (SIBPAL, or Sib-Pair Linkage Program, Version 2.6, authored by Tran, L.D., et al., Louisiana State University Medical Center, New Orleans, LA) (the program package S.A.G.E. (1994), or Statistical Analysis for Genetic Epidemiology, Release 2.2, Dept. Of Biometry and Genetics, LSU Medical Center, is supported by a U.S. Public Health Service Resource Grant 1 P41 RR03655, from the Division of Research Resources).

Among 47 affected sibpairs, 44 share one or two TNF microsatellite haplotypes, which is greater than the expected value of 35.25 at the 0.005 significance level. The results of SIBPAL analysis also are consistent with linage. Mean proportion of haplotypes shared identical by descent in: affected concordant sibpairs are m=0.62, p=0.004 (n=47); clinically discordant sibpairs are m=0.42, p=0.02 (n=72); and, unaffected concordant sibpairs, m-0.56, p=0.11 (n=52). Furthermore, linear regression analysis of the phenotypic difference versus proportion of haplotypes shared identical-by-decent yielded a slope of -0.35, p-0.0007. The vertical axis represents the difference between the phenotypes between the sibs. So if the sibs have the same phenotype the difference is zero, if they have different phenotypes the difference is one. So if there is linkage between disease and the marker (e.g. gene, allele), the difference would be small (approaching zero). All these results, *i.e.* the negative slope, are consistent with identifying a statistically significant negative slope consistent with (demonstrating) a linkage between CD and TNF locus. See Yang, H., *et al.*, *Am. J. Human Genetics* 57:A233, abst. 1348 (1995), which is herein incorporated by reference.

In conclusion, an analysis of 29 multiplex families by several nonparametric linkage methods support the linkage of CD to the TNF locus.

EXAMPLE II

To demonstrate that TNF levels in CD have a genetic component, experiments determined that there is a

heterogeneity in TNF production between UC and CD patients and between different allelic combinations, or haplotypes, of CD. As shown in Figure 2, total TNF production from lectin (Con A) and phorbol ester-activated PBMC of CD patients is as a whole greater than TNF production in UC patients. Additionally, the TNF haplotype A2B1C2D4E1 correlates with increases in TNF-α protein production in patients with CD.

The present invention demonstrates that the TNF haplotype A2B1C2D4E1 defines a subtype of CD. Total TNF and TNF-α bioactivity were measured at various time points using PBMC from CD and UC patients. The PBMC were activated with optimal concentrations of LPS, PHA and concanavalin A (Con A) plus the phorbol ester phorbol myristic acid (PMA). PBMC are isolated from peripheral blood using Percoll Gradient and immediately cultured at $2x10^6$ cells per ml. in RPMI with 10% fetal calf serum (FCS). Cell are activated with Con A at 20 ngm per ml., PMA at 2 ng per ml, LPS at 10 ugm per ml. and PHA at 10 ugm per ml. TNF-α and TNF-β bioactivity (*i.e.*, the ability to kill cells) is determined at intervals from 24 to 60 hours. Following activation, the cells are isolated and then stored in aliquots until assayed at minus 70°C. Total TNF bioactivity is determined by L929 fibroblast cytotoxicity assay.

As shown in Figure 2A, TNF production from PBMC activated with Con A and PMA for 24 hours was higher in patients with CD than UC. TNF production from UC patients was about 10^3 picograms per ml, while TNF production from CD patients was about $4x10^3$ picograms per ml. For the UC sample n=20 and for the CD sample n=27, with p=0.02. Other stimuli showed no differences, suggesting that responsiveness to phorbol ester can define a unique characteristic in CD.

Although the CD group as a whole had a greater TNF secretion than the UC group, as demonstrated in Figure 2A, there was a heterogeneous TNF response amongst the group of patients with CD. As shown in Figure 2B, the subgroup of CD patients with the TNF allelic combination (haplotype) a2b1c2d4e1 appeared to account for the increased TNF production in CD. TNF

production from CD patients lacking haplotype a2b1c2d4e1 was about $2x10^3$ picograms per ml, while TNF production from CD patients with haplotype a2b1c2d4e1 was about $4x10^3$ picograms per ml. For the non-a2b1c2d4e1 haplotype sample n=20 and for the a2b1c2d4e1 haplotype sample n=7, with p=0.12.

Since it was not known which of the alleles associated with this allelic combination is associated with increased TNF production, results from patients who had the TNF haplotype a2b1c2d4e1 were compared to the results from patients who had none of the individual alleles. As shown in Figure 2C, this comparison defined a more homogeneous subtype of patient that had increased TNF responses to Con A and PMA stimulation. TNF production from CD patients without any alleles from the haplotype a2b1c2d4e1 was about $1x10^3$ picograms per ml, while TNF production from CD patients with haplotype a2b1c2d4e1 was about $4x10^3$ picograms per ml. For the null-a2b1c2d4e1 haplotype sample n=9 and for the a2b1c2d4e1 haplotype sample n=7, with p=0.04.

In conclusion, the CD group as a whole had a greater TNF secretion than the UC group. The subgroup of CD patients with the TNF allelic combination a2b1c2d4e1 appeared to account for the increased TNF production in CD. Furthermore, these data support a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The method would comprise identifying a TNF locus allelic combination, such as the TNF allelic combination a2b1c2d4e1, by analyzing a nucleic acid from a subject diagnosed with having an inflammatory bowel disease. Confirming the presence of an allelic combination known to have a correlation with increased levels of TNF-α and IBD allows an evaluation of the effectiveness of the therapy. The evaluation would effect diagnosis, selection of patients, election of treatment modalities and prognosis after treatment to decrease TNF levels.

EXAMPLE III

To further demonstrate that TNF levels in CD patients have a genetic component, experiments determined that a particular allele within an allelic combination, or haplotype, is associated with increased TNF production in patients with CD.

To determine if there was a particular allele within the TNF haplotype a2b1c2d4e1 that could be more closely associated with increased TNF production, the data was analyzed by stratifying TNF production with the individual TNF alleles. As the experimental results in Figure 3 demonstrate, the presence of the d4 allele correlated with high TNF production compared to those patients who did not express d4. The TNF "d" locus is located downstream (5') of the TNF-α gene.

In conclusion, the increased TNF-α production in a subset of CD patients also correlated with the presence of the TNF allelic combination a2b1c2d4e1, and particularly the d4 allele expressed in the "d" locus. Furthermore, these data support a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The method would comprise identifying a TNF allele, such as the d4 allele, by analyzing a nucleic acid from a subject diagnosed with having an inflammatory bowel disease. Confirming the presence of an allele known to have a correlation with increased levels of TNF-α and IBD allows an evaluation of the effectiveness of the therapy. The evaluation would effect diagnosis, selection of patients, election of treatment modalities and prognosis after treatment to decrease TNF levels.

EXAMPLE IV

To demonstrate that the TNF haplotype a2b1c2d4e1 is not linked to all TNF-α dysregulated allelic variations in CD patients, additional allelic variations were identified in CD patients.

TNF polymorphisms exist in functionally significant regions of the TNF locus. Two polymorphisms have been described in the 5' regulatory region of the human TNF-α gene. One has guanosine nucleotide substitution for adenosine at the -238

position and another allele has the same substitution at the -308 position. These single base changes were analyzed in eight CD patients with the TNF haplotype a2b1c2d4e1. It was found that neither of the guanosine nucleotide substitution alleles is linked to the a2b1c2d4e1 haplotype. Polymorphisms were determined by dot-blot of genomic DNA with hybridization of P^{32} 5'-end labeled allele-specific oligonucleotides, as described in Plevy, S.E., *et al.*, PCT US95/06107, WO 95/31575, which is herein incorporated by reference in its entirety. As the results in Figure 4 demonstrate, zero out of eight CD patients with the TNF a2b1c2d4e1 haplotype have the -238 allele and only one of eight patients have the -308 allele.

In conclusion, these results demonstrate novel methods to define new polymorphisms in TNF locus gene regulatory regions. Furthermore, these data support a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The method would comprise identifying a TNF polymorphism, such as the TNF-α guanosine nucleotide substitution for adenosine at the -238 position and the -308 position, by analyzing a nucleic acid from a subject diagnosed with having an inflammatory bowel disease. Confirming the presence of a TNF polymorphism known to have a correlation with increased levels of TNF-α and IBD allows an evaluation of the effectiveness of the therapy. The evaluation would effect diagnosis, selection of patients, election of treatment modalities and prognosis after treatment to decrease TNF levels.

****

Although the invention has been described with reference to the examples above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

CLAIMS

1. A method of identifying an inflammatory bowel disease or a subtype thereof in a subject comprising:

detecting a polymorphism at a TNF locus by analyzing a nucleic acid from the subject, wherein said polymorphism does not constitute a TNF locus microsatellite allele;

identifying a biological response related to the inflammatory bowel disease; and,

identifying a correlation between said polymorphism and the biological response.

2. A method of diagnosing an inflammatory bowel disease or subtype thereof in a subject comprising:

detecting a polymorphism at a TNF locus by analyzing a nucleic acid from a subject, wherein said polymorphism does not constitute a TNF locus microsatellite allele; and

using the correlation identified by the method of claim 1 to diagnose the presence of the inflammatory bowel disease or subtype thereof.

3. A method of screening for a susceptibility to an inflammatory bowel disease or subtype thereof in a subject comprising:

detecting a polymorphism at a TNF locus by analyzing a nucleic acid from a subject, wherein said polymorphism does not constitute a TNF locus microsatellite allele; and

using the correlation identified by the method of claim 1 to evaluate the susceptibility of the subject for the inflammatory bowel disease or subtype thereof.

4. The method of claim 1, 2, or 3, wherein said polymorphism comprises any nucleotide substitution, addition, deletion or combination thereof.

5. The method of claim 4, wherein said nucleotide substitution comprises a substitution in the 5' regulatory region of a TNF-∝ locus.

6. The method of claim 4, wherein said nucleotide substitution in said TNF-∝ locus 5' regulatory region is selected from the group consisting of a guanosine nucleotide substitution for adenosine at the -238 position and a guanosine nucleotide substitution for adenosine at the -308 position.

7. The method of claim 1, 2, or 3, wherein said inflammatory bowel disease comprises Crohn's Disease.

8. The method of claim 1, 2, or 3, wherein said TNF locus comprises a TNF-∝ locus.

9. The method of claim 1, wherein said biological response comprises an increase in the expression of cytokine.

10. The method of claim 9, wherein said cytokine comprises TNF-∝.

11. A method of screening for a susceptibility to Crohn's Disease in a subject comprising identifying a polymorphism in a 5' regulatory region of a TNF-∝ locus by analyzing a nucleic acid from the subject, wherein said polymorphism is selected from a guanosine nucleotide substitution for adenosine at the -238 position or a guanosine nucleotide substitution for adenosine at the -308 position.

12. A method of determining whether a therapy which decreases the levels of TNF-∝ would be effective in treating an inflammatory bowel disease comprising:

detecting a TNF locus polymorphism by analyzing a nucleic acid from a subject, wherein said polymorphism does not constitute a TNF locus microsatellite allele; and

using the correlation found by the method of claim 1 to evaluate the effectiveness of the therapy.

13. The method of claim 12, wherein the inflammatory bowel disease comprises Crohn's Disease.

14. The method of claim 12, wherein the TNF locus polymorphism comprises any nucleotide substitution, addition, deletion or combination thereof.

15. The method of claim 14, wherein said nucleotide substitution comprises a substitution in the 5' regulatory region of a TNF-∝ locus.

16. The method of claim 15, wherein said nucleotide substitution in said TNF-∝ locus 5' regulatory region is selected from the group consisting of a guanosine nucleotide substitution for adenosine at the
-238 position and a guanosine nucleotide substitution for adenosine at the -308 position.

17. An assay system for screening for a susceptibility to inflammatory bowel disease in a subject comprising nucleic acid encoding a 5' regulatory region of a TNF-∝ locus that includes position -238, or complementary sequence thereto.

18. The assay system of claim 17 further comprising means for amplifying the 5' regulatory region of a TNF-∝ locus that includes position -238.

19. An assay system for screening for a susceptibility to inflammatory bowel disease in a subject comprising nucleic acid encoding a 5' regulatory region of a TNF-∝ locus that includes the position -308, or complementary sequence thereto.

20. The assay system of claim 19 further comprising means for amplifying the 5' regulatory region of a TNF-∝ locus that includes position -308.

21. The method of claim 1, 2, 3 or 12, wherein said detecting comprises:

(a) obtaining a sample from a subject;

(b) preparing a nucleic acid comprising TNF locus DNA from said sample;

(c) contacting said nucleic acid with a polymorphism-specific oligonucleotide probe under conditions suitable for formation of a specific hybrid between said nucleic acid and said polymorphism-specific oligonucleotide probe; and

(d) assaying for the presence of said specific hybrid, wherein the presence of said specific hybrid indicates said polymorphism.

22. The method of claim 21, wherein preparing said nucleic acid comprises enzymatic amplification of said nucleic acid.

23. The method of claim 21, wherein said polymorphism-specific oligonucleotide probe comprises a sequence of a 5' regulatory region of a TNF-∝ locus that includes position -238, or complementary sequence thereto.

24. The method of claim 21, wherein said polymorphism-specific oligonucleotide probe comprises a sequence of a 5' regulatory region of a TNF-α locus that includes position -308, or complementary sequence thereto.

25. The method of claim 21, wherein preparing said nucleic acid further comprises enzymatic restriction digestion of said nucleic acid.

FIG. 1

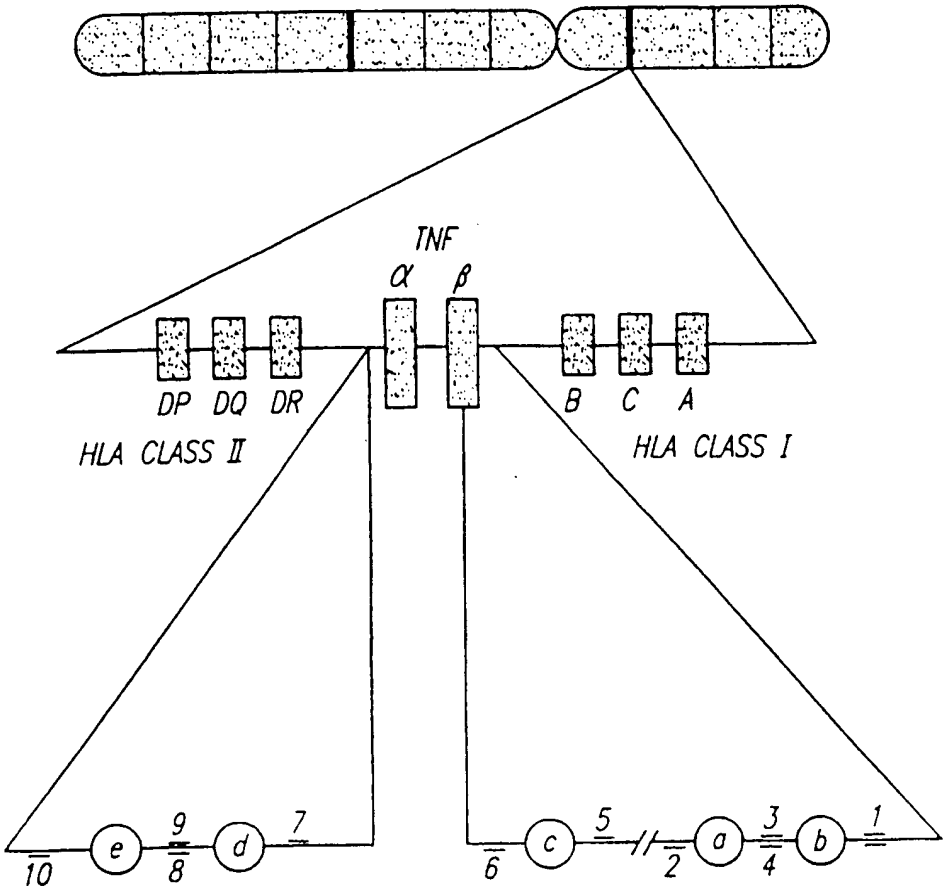
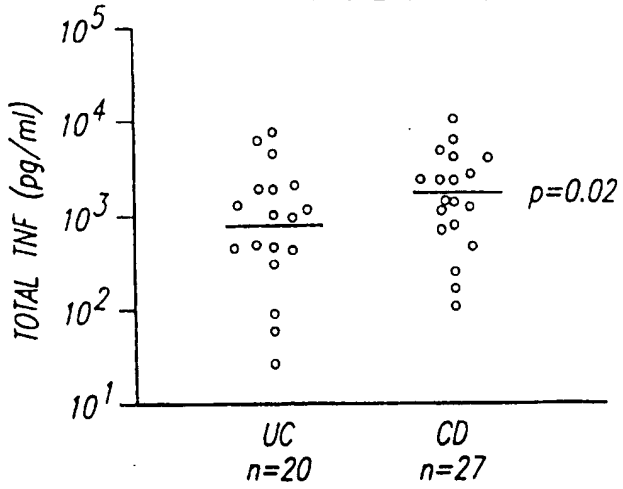


FIG. 2A



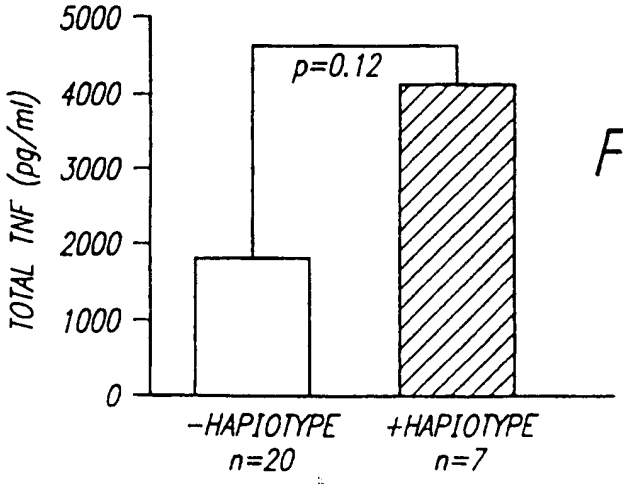


FIG. 2B

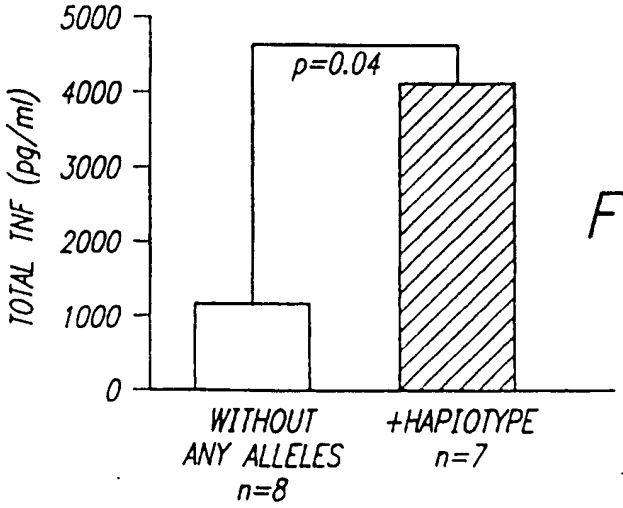


FIG. 2C

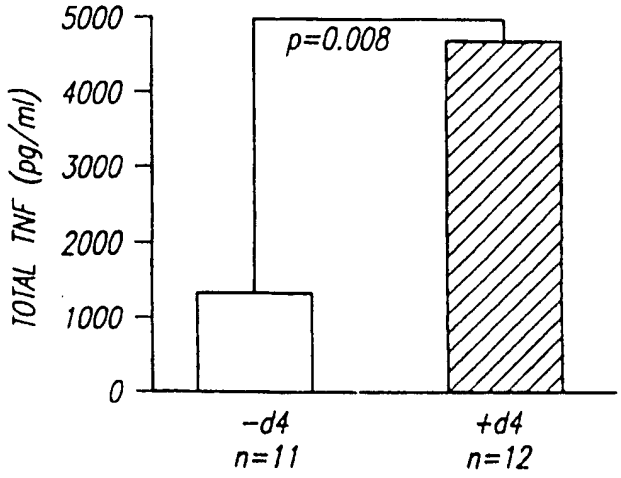


FIG. 3

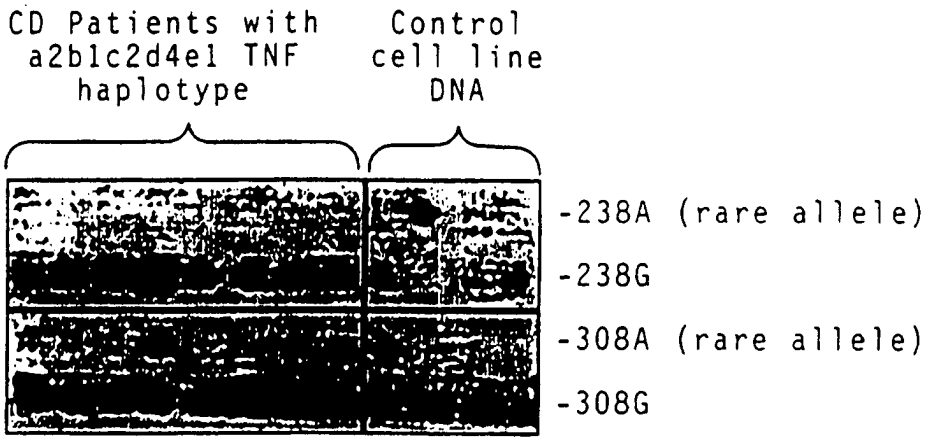


FIG. 4

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 97/06039

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCANDINAVIAN JOURNAL OF IMMUNOLOGY, vol. 43, no. 4, April 1996, pages 456-63, XP002038831 BOUMA G ET AL: "Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-D alleles. Relevance for inflammatory bowel disease" see page 462, paragraph 2 ---	1-25
A	CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 103, no. 3, March 1996, pages 391-6, XP002038832 BOUMA G ET AL: "Distribution of four polymorphisms in the tumour necrosis factor (TNF) genes in patients with inflammatory bowel disease (IBD)" see abstract and discussion. --- -/--	1-25

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 August 1997	09. 09. 97

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 31575 A (CEDARS SINAI MEDICAL CENTER) 23 November 1995	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

national Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9531575 A	23-11-95	AU 2638495 A	05-12-95
		CA 2190586 A	23-11-95
		EP 0760010 A	05-03-97

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Published
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With amended claims.

Date of publication of the amended claims:
11 December 1997 (11.12.97)

(54) Title: METHODS OF IDENTIFYING AND DIAGNOSING INFLAMMATORY BOWEL DISEASE

(57) Abstract

The present invention provides methods of identifying, diagnosing and screening for inflammatory bowel disease, particularly Crohn's Disease (CD) comprising identifying alleles and polymorphisms associated with a biological response related to an inflammatory bowel disease. The invention further provides for a method of determining whether a therapy which decreases the levels of TNF-α would be effective in treating an inflammatory bowel disease. The invention also provides for an assay system for screening for susceptibility to inflammatory bowel disease.

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AMENDED CLAIMS

[received by the International Bureau on 23 October 1997 (23.10.97);
original claims 1-25 amended; claim 26 added (5 pages)]

1. A method of identifying an inflammatory bowel disease (IBD) or subtype thereof, comprising

selecting a population comprised of subjects having at least one biological response associated with IBD or a subtype thereof;

obtaining nucleic acid from the subjects in the selected population;

detecting a polymorphism at a functional site of a tumor necrosis factor (TNF) locus in the nucleic acid from the subjects;

establishing whether a statistically significant correlation exists between the thus found polymorphism and at least one of the biological responses; and

identifying an IBD or subtype thereof when the existence of a statistically significant correlation is established.

2. A method of diagnosing an inflammatory bowel disease (IBD) or subtype thereof, comprising

selecting a subject exhibiting at least one biological response associated with IBD of subtype thereof;

detecting a polymorphism at a functional site of a tumor necrosis factor (TNF) locus in the nucleic acid from the subject;

comparing the found polymorphism with that of IBD or subtype DNA controls found by the method of claim 1 to have a statistically significant correlation with a biological response; and

diagnosing IBD or a subtype thereof when a match with an IBD or subtype control is found.

3. A method of screening a population for susceptibility to inflammatory bowel disease (IBD) or subtype thereof, comprising

diagnosing IBD or subtype thereof by applying the method of claim 2 to nucleic acid obtained from members of a predetermined population; and

selecting the members of the population which show a statistically significant correlation

with the biological response as exhibiting a susceptibility to IBD or subtype thereof.

4. The method of claim 1, wherein the polymorphism comprises a mutation selected from the group consisting of nucleotide substitutions, additions, deletions and combination thereof.

5. The method of claim 4, wherein

the polymorphism comprises a nucleotide substitution; and

the nucleotide substitution comprises a substitution in the 5' regulatory region of a TNF-α locus.

6. The method of claim 5, wherein the nucleotide substitution in the TNF-α locus 5' regulatory region is selected from the group consisting of guanosine nucleotide substitutions at positions -238 and -308.

7. A method of identifying Crohn's Disease, comprising the method of claim 1, wherein the IBD comprises Crohn's Disease.

8. The method of claim 1, wherein the TNF locus comprises a TNF-α locus.

9. The method of claim 1, wherein the biological response comprises an increase in the expression of the TNF-α gene with respect to a general population, as measured by TNF-α protein production or blood levels .

10. The method of claim 1, wherein the polymorphism is detected by

isolating a fragment of the nucleic acid comprising the TNF locus from the sample;

contacting the nucleic acid fragment with a TNF polymorphism-specific oligonucleotide probe under conditions effective to hybridize the nucleic acid and the polymorphism-specific oligonucleotide probe;

detecting the presence of any hybrid formed; and

taking the presence of any hybrid as an indication of the existence of a polymorphism.

11. The method of claim 10, wherein the nucleic acid is isolated by enzymatic amplification.

12. The method of claim 10, wherein the TNF polymorphism-specific probe comprises a TNF locus 5' regulatory region oligonucleotide selected from the group of oligonucleotides comprising residue -238 and oligonucleotides complementary thereto.

13. The method of claim 10, wherein the TNF polymorphism-specific probe comprises a TNF locus 5' regulatory region oligonucleotide selected from the group consisting of oligonucleotides comprising residue -308 and oligonucleotides complementary thereto.

14. The method of claim 10, further comprising enzymatically restricting the nucleic acid.

15. A method of diagnosing a susceptibility to Crohn's Disease (CD) in a subject, comprising identifying a polymorphism in a subject's DNA at a 5' regulatory region of a tumor necrosis factor-α (TNF-α) locus selected from the group consisting of positions -238 and -308; and

diagnosing the subject as having a susceptibility to CD if either position comprises a guanosine nucleotide substitution.

16. A method of determining the effectiveness of a therapy to decrease the serum level of tumor necrosis factor-α (TNF-α) for treating inflammatory bowel disease, comprising

detecting a polymorphism at a functional site within a TNF-α locus in a nucleic acid from a subject; and

determining that the therapy is effective using the correlation found by the method of claim 1 with respect to TNF-α blood levels, if the TNF-α blood level is decreased and no longer exhibits a statistically significant correlation with the polymorphism.

17. The method of claim 16, wherein the inflammatory bowel disease comprises Crohn's Disease.

18. The method of claim 16, wherein the TNF locus polymorphism comprises a mutation selected from the group consisting of nucleotide substitutions, additions, deletions and combinations thereof.

19. The method of claim 18, wherein the mutation comprises a nucleotide substitution; and the nucleotide substitution comprises a substitution in the 5' regulatory region of a TNF-α locus.

20. The method of claim 19, wherein the nucleotide substitution in 5' regulatory region of the TNF-α locus is selected from the group consisting of a guanosine substitution at position -238 and -308.

21. An inflammatory bowel disease kit, comprising

a nucleic acid comprising a DNA selected from the group consisting of those encoding a 5' regulatory region of a TNF-α locus comprising position -238 and complementary sequences thereto; and

instructions for its use.

22. The kit of claim 21, further comprising means for amplifying the nucleic acid.

23. The kit of claim 21, wherein the DNA is about 10 to 30 nucleotide long.

24. An inflammatory bowel disease kit, comprising

a nucleic acid comprising a DNA selected from the group consisting of those encoding a 5' regulatory region of a TNF-α locus comprising position -308 and complementary sequences thereto; and

instructions for its use.

25. The kit of claim 24, further comprising means for amplifying the nucleic acid.

26. The kit of claim 24, wherein the DNA is about 10 to 30 nucleotide long.